OIPE JC10
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EXPRESS MAIL NO. EV462737349US		
DECLARATION OF ROSS STEIN UNDER 37 C.F.R. § 1.132 Address to: Commissioner for Patents Alexandria, VA 22313-1450	Attorney Docket Confirmation No.	UCAL161DIV 7273
	First Named Inventor	Steven Finkbeiner
	Application Number	09/922,483
	Filing Date	August 2, 2001
	Group Art Unit	1648
	Examiner Name	U. Winkler
	Title	*Antibodies specific for proteins having polyglutamine expansions*

Dear Sir:

1. I, Ross Stein declare and say I am an expert in the field of enzyme kinetics and drug discovery. I currently am affiliated with the Brigham and Women's Hospital in the Department of Neurology, the Laboratory for Drug Discovery in Neurodegeneration (LDDN). A copy of my curriculum vitae is provided herewith as Exhibit 2.

2. I understand that certain of the currently pending claims in the above-captioned patent application recite a screening method that assays the ability of a test agent to inhibit binding between an antibody specific for a polyglutamine expansion and a protein containing such a polyglutamine expansion. The screening method identifies agents that modulate the binding interaction between a protein comprising a polyglutamine expansion and a cellular target of such a protein.

3. I understand that a United States Patent and Trademark Office Examiner has rejected such claims on the basis that measuring the interaction between a polyglutamine expansion-containing protein and an antibody specific for a polyglutamine expansion will not provide any insight into the interaction of the polyglutamine expansion-containing protein and its cellular target.

4. In my opinion, it is reasonable to use such an assay to identify agents that inhibit binding of a protein containing a polyglutamine expansion to its cellular target. The antibody that will be used in this assay has a specific binding site for a toxic conformation of polyglutamine. It is not unreasonable to assume that the binding site on the antibody might have structural features and binding properties for polyglutamine that are similar to binding sites on the cellular proteins that mediate the toxic effects of polyglutamine. Therefore, small drug-like molecules that bind to the site on the antibody, thereby blocking polyglutamine from binding to the antibody, might also bind to these cellular proteins. I believe that this type of assay represents an important and valuable approach to identifying new therapeutic agents for neurodegenerative disorders.

5. I am principle investigator on a grant application submitted to the National Institute of Neurological Disorders and Stroke (NINDS) that includes a collaboration with Dr. Steven Finkbeiner and the use of as this assay as a key component. The grant application included the proposed use of such an assay in a high throughput screen format to identify agents that inhibit binding of a protein containing a polyglutamine expansion to its cellular target. This grant was reviewed be an NINDS Special Emphasis panel on April 23, 2004. The comments of the panel were strongly supportive of the science of the grant, including this assay.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code, and that such will false statements may jeopardize the validity of the application or any patent issuing thereon.

7/14/04
Date

Ross Stein

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PART I: General Information

Date Prepared: 03/10/2004

Name: Ross Lee Stein, Ph.D.

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Home Address: 14 Hawes Road
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Place of Birth: St. Louis, MO

Education

Year	Degree	Institution
1974	B.S., Chemistry	Southern Illinois University Edwardsville, IL
1978	Ph.D., Biochemistry	Indiana University Bloomington, IN

Postdoctoral Training

Year	Title	Place of Training	Specialty/Discipline
1978 - 1979	Postdoctoral Fellow	Washington University School of Medicine St. Louis, MO	Enzymology/Pharmacology
1979 - 1981	Research Associate	University of Kansas Lawrence, KS	Mechanistic Enzymology

Professional Positions

Year	Position/Title	Institution
1981 – 1984	Research Biochemist Pharmacology Department	Stuart Pharmaceuticals Wilmington, DE
1984 – 1987	Senior Research Biochemist Pharmacology Department	Stuart Pharmaceuticals Wilmington, DE
1987 - 1989	Associate Director Department of Enzymology	Merck Research Labs Rahway, NJ
1989 - 1992	Director Department of Enzymology	Merck Research Labs Rahway, NJ
1992 - 1993	Director Enzymology	Genesis Pharmaceuticals Cambridge, MA
1993 - 1995	Executive Director Biochemistry	ProScript, Inc. Cambridge, MA
1995 - 1998	Vice President Biochemistry	ProScript, Inc. Cambridge, MA
1998	Senior Director Biochemistry	Cubist Pharmaceuticals Cambridge, MA
1999 – 2001	Research Fellow Chemical Enzymology	DuPont Pharmaceuticals Wilmington, DE
2001 - present	Director Laboratory for Drug Discovery in Neurodegeneration	Harvard Medical School Boston, MA
2003 - present	Associate Professor Department of Neurology	Harvard Medical School Boston, MA

Professional Societies

Year	Society	Role
1972 - present	American Chemical Society	Member
1976 - present	FASEB	Member
1981 - present	AAAS	Member

PART II: Research, Teaching, and Clinical Contributions

Summary of Research and Managerial Contributions

My research contributions have been in the areas of mechanistic enzymology and drug discovery. I have contributed to the understanding of mechanisms of catalysis of several enzymes, including: purine nucleoside phosphorylase, elastase, stromelysin, peptidyl prolyl *cis-trans* isomerase, the proteasome, ubiquitin C-terminal hydrolase, signal peptidase, γ-glutamyl transpeptidase, penicillin binding proteins, and aryl acylamidase. In all cases, I have applied precise kinetics-based probes to elucidate key structural features of catalytic transition states. The knowledge gained from these studies has been applied to the design and mechanistic characterization of inhibitors of these enzymes and, in several cases, to the development of new therapeutics for human disease. For elastase and the proteasome, these basic mechanistic studies contributed in a direct way to the development of a drug that was in clinical trials in the early 1990's for cystic fibrosis (i.e., elastase inhibitor) and a drug, VELCADE^{TM}, that has been approved by the FDA for the treatment of certain cancers (i.e., proteasome inhibitor).

As a manager, I have built and run research departments in both pharmaceutical and biotech companies. At Merck, I built the Department of Enzymology from seven scientists to about twenty five. This department contributed broadly to many projects at Merck that had enzymes as their therapeutic targets. Several of these projects resulted in drugs that went into human clinical trials. At ProScript, I was the company's first scientist and helped build the research department to about thirty scientists. As head of Biochemistry, I managed a staff that worked on the development of assays and elucidation of mechanism for enzymes of the ubiquitin-proteasome pathway. These enzymes include the proteasome, ubiquitin activating enzyme, ubiquitin conjugating enzymes, and ubiquitin C-terminal hydrolases. The work of this group of scientists was instrumental in the development of a proteasome inhibitor that is now in clinical trials.

Current Research and Managerial Activities

My current activities as Director of the Laboratory for Drug Discovery in Neurodegeneration include hiring staff, managing the day-to-day operation of the facility, working with HMS staff and post-doctoral fellows to develop and optimize assays to be run as high-throughput screens, fund-raising activities, and conducting mechanistic studies on the compounds that are discoved in the various screens.

My research interests are in the areas of enzyme catalysis and inhibition. I am concerned with trying to understand the mechanistic origins of the enormous rate-accelerations that are effected by enzymes and how knowledge gained about enzymatic catalysis can be used to design inhibitors and understand how enzyme inhibitors work. Specific to these interests, I am currently undertaken two projects: acylation chemistry and dynamics of serine hydrolases and mechanisms of transglutaminase-catalyzed transamidation.

Research Funding

"Harvard Center for Neurodegeneration and Repair Core D" 01/01/01 – 12/31/05
Principal Investigator: Peter T. Lansbury, Jr., Ph.D.
Agency: Harvard Medical School
The goals of Core D of the Harvard Center for Neurodegeneration and Repair are to discover new drugs for the treatment of neurodegneerative diseases and to discover tool-compounds that can be used to probe pathogenesis incell- and animal-models of disease. This project is being done in the Laboratory for Drug Discovery for Neurodegeneration, which was created as the means to best attain the overarching goals of Core D. The ongoing activities of the LDDN include the development and execution of novel cell-, enzyme-, and receptor-based assays for multiple disease targets. These assays are run against an in-house library of approximately 52,000 compounds. This project also includes medicinal chemistry-directed optimization of active compounds that are discovered during the screening process.

"Inhibition of antigen Presentation in Multiple Sclerosis" 12/1/02 – 11/30/06
Principal Investigator: Kai W. Wucherpfenning, M.D., Ph.D.
Agency: NIH
The specific aims of the project are: To develop robust assays for high-throughput screening of small molecules that block presentation of myelin peptides (Aim 1), to screen a large and diverse collection of small molecules under conditions relevant for intracellular peptide loading (Aim 2), and to study the mechanisms by which lead compounds inhibit presentation of myelin peptides (Aim 3). We are in a unique position to pursue this project, based on our combined expertise in immunology and drug discovery, and the preliminary data demonstrate that both assay development and high throughput screening are feasible.

"Chemical Genetics and Regulated Intramembrane Proteolysis" 7/1/03 – 6/30/08
Principal Investigator: Michael S. Wolfe, Ph.D.
Agency: NIH
The goal of this project is to apply the princiaples of chemical genetics to discover bio-organic agenst suitable of the studh of N and APP processing in signaling, developmental biology and human disease and to determine the protein targets of these agents.

"Metabolic Pathways and Defects in Fructose Metabolism" 7/1/03 – 6/30/08
Principal Investigator: Dean R. Tolan, Ph.D.
Agency: NIH
The major goals of this project are to 1) define sites for fructose assimilation and utilization using a combination of bioinformatics and molecular approaches, 2) determine a high-resolution structure of AP-aldolase, and use it to find stabilizing small-molecule ligands by both structure-based ligand design (SBLD) and using combinatorial chemistry, 3) create animal models for HFI using gene-targeting techniques, and 4) identify HFI mutations in the diverse US population, in particular Hispanic, African-American, and other ethnic groups that have not been well characterized, and correlate these findings to any specific phenotypes in these ethnic groups

"Compound Identification in Assays for Tau Pathology" 2/1/03 – 1/31/05
Principal Investigator: Kenneth S. Kosik, M.D.
Agency: NIH
Period: 2/1/03 – 1/31/05
The goal of this project is to develop tow high through-put screens aimed at the discovery of compounds with a potential impact on neurodegenerative pathologies related to tau

Teaching Responsibilities

My teaching responsibilities include formal and informal lectures, and the mentoring of post-doctoral students and technicians in the areas of enzyme kinetics, enzyme mechanism, drug discovery, and high throughput screening. Among the lectures are formal presentations at the Center for Neurologic Diseases (CND), Brigham and Women's Hospital and a lecture series on enzyme kinetics and mechanism given within the Laboratory for Drug Discovery in Neurodegeneration (LDDN). These lectures include the following:

"Role of Mechanistic Studies in Drug Discovery", CND, June, 2001.

"Principles of High-Throughput Screening", LDDN, September, 2001.

"Role of Mechanistic Studies in Drug Discovery – Assay Development", LDDN, October, 2001.

"Role of Mechanistic Studies in Drug Discovery – Kinetic Analysis of Enzyme Inhibitors", LDDN, December, 2001.

"Role of Mechanistic Studies in Drug Discovery – Principles of Elucidating Enzyme Mechanisms for Catalysis and Inhbition. I", LDDN, February, 2002.

"Role of Mechanistic Studies in Drug Discovery – Principles of Elucidating Enzyme Mechanisms for Catalysis and Inhbition. II", LDDN, May, 2002.

"Tranglutaminase as a Target for Huntington's Disease – Mechanism, Inhbitor Design, and High-Throughput Screenin", LDDN, February, 2003.

Mentoring has included numerous sessions with many post-docs, including:

Melissa Nicholson (Wucherpfennig lab, DFCI) – kinetics of receptor binding

Craig Justman (Lansbury lab, BWH) – mechanism of synuclein aggregation

Jae Ahn (Kosik lab, BWH) – kinase kinetics and screening for inhibitors

Malcolm Leissring (Selkoe lab, BWH) – kinetics of protease action

Yichin Liu (Lansbury lab, BWH) – kinetics and mechanism of ubiquitin hydrolases

David Wilson (Stein lab, LDDN) – kinetics and mechanism of tau aggregation

April Case (Stein lab, LDDN) – kinetics and mechanism of transglutaminase

Teaching Experience – Invited Regional, National and International Contributions

1. "Catalysis by Human Leukocyte Elastase." Presented to the Department of Chemistry, University of Kansas, July 1982.

2. "Catalysis by Human Leukocyte Elastase." Presented to the Department of Chemistry, Southern Illinois University, December 1982.

3. "An Automated System for Enzyme Kinetics." Presented at the First International Symposium on Laboratory Robotics, October 1983.

4. "Catalysis by Human Leukocyte Elastase." Presented to the Department of Chemistry, Georgia Institute of Technology, January 1984.

5. "Role of Subsite Interactions in Catalysis by Human Leukocyte Elastase." Presented to the Department of Pharmacology, Institute for Medical Research, Louis Pasteur University, Strasbourg, France, October, 1984.

6. "Role of Subsite Interactions in Catalysis by Human Leukocyte Elastase." Presented to the Department of Chemistry, Southern Illinois University, October, 1984.

7. "Mechanism of Action of Human Leukocyte Elastase." N.I.H. Workshop on Elastase Inhibition, June 10-11, 1985.

8. "Mechanism of Action of Human Leukocyte Elastase." Presented to the Department of Chemistry, University of Kansas, October1985.

9. "Mechanism of Action of Human Leukocyte Elastase." Presented to the Department of Chemistry, Wichita State University, October 1985.

10. "The Proton Inventory as a Probe of Protease Mechanism." Gordon Research Conference on Isotopes, February 1986.

11. "Mechanisms of Slow-Binding Inhibition of Serine Proteases." Presented to the Department of Chemistry, University of Kansas, November 1987.

12. "Mechanistic Insights into the Substrate Specificity of Human Leukocyte Elastase." Presented to the Department of Chemistry, University of Kansas (1987).

13. "Mechanisms of Slow-Binding Inhibition of Serine Proteases." Midwest American Chemical Society Meeting, Wichita State University, November 1987.

14. "Mechanisms of Slow-Binding Inhibition of Serine Proteases." Presented to the Department of Biochemistry, Tufts University, December 1987.

15. "Mechanisms of Slow-Binding Inhibition of Serine Proteases." Presented to the Department of Chemistry, University of Iowa, April 1988.

16. "Mechanistic Studies of Metalloproteases." Presented to the Department of Chemistry, Texas A&M University, March 1989.

17. "Mechanistic Studies of Metalloproteases." Presented to the Department of Biochemistry, University of Washington, April 1989.

18. "Mechanistic Studies of Peptidyl Prolyl cis-trans Isomerase." Presented to the Department of Chemistry, Florida State University, April 1990.

19. "Mechanistic Studies of Peptidyl Prolyl cis-trans Isomerase." Presented to the Medical School, Louis Pasteur University, Strasbourg, France, October 1990.

20. "Studies of the Matrix Metalloproteinase, Stromelysin." Presented to the Dept. of Pharmacy, Louis Pasteur University, Strasbourg, France, October 1990.

21. "The Proton Inventory as a Probe of Enzyme Mechanism." Presented to the Dept. of Pharmacy, Louis Pasteur University, Strasbourg, France, October 1990.

22. "Mechanistic Studies of Peptidyl Prolyl cis-trans Isomerase." Presented at Cyclosporin, Immunosuppression and Protein Folding, an international meeting held in Brandenburg, Germany, October 1990.

23. "Mechanistic Studies of Peptidyl Prolyl cis-trans Isomerase." Department of Biophysics, Boston University Medical School, November 1990.

24. "Mechanistic Studies of Peptidyl Prolyl cis-trans Isomerase." Department of Chemistry, Weslyan University, September 1990.

25. "Mechanistic Studies of Stromelysin Catalysis and Inhibition." International Conference on Cartilage Destruction and Repair, Redbank, NJ, September 1991.

26. "Solvent and Secondary Kinetic Isotope Effects as Probes of Protease Catlaysis and Inhibition." Gordon Conference on Isotope Effects in the Physical and Life Sciences, Ventura, CA, March 1992.

27. "Mechanistic Studies of Metalloproteinase Catalysis and Inhibition" Gordon Conference on Proteolytic Enzymes, Plymouth, N.H., June 1992

28. "Mechanistic Studies of Peptidyl Prolyl cis-trans Isomerase." Symposium on Enzymes, Inhibitors and Drug Design", Antwerp, Belgium, November 1992.

29. "Inhibition of Proteasome-Dependent Activation of NF-kB: A Novel Strategy for the Development of Anti-Inflammatory Agents", SRI Symposium: On the Cutting Edge of Anti-Inflammatory Drug Discovery", February 1996.

30. "Novel Inhibitors of the Proteasome", Gordon Conference on Proteolytic Enzymes, Plymouth, N.H., July 1996.

31. "Inhibition of Proteasome-Dependent Activation of NF-κB: A Novel Strategy for the Development of Anti-Inflammatory Agents", IBC's 2nd International Conference on Proteases Inhibitors, Washington, D.C. February 1997.

32. "Enzymology and Inhibition of the Ubiquitin-Proteasome Pathway", Gordon Research Conference on Enzymes, Coenzyme, and Metabolic Pathways, Kimball Union Academy, N.H., July, 1998.

33. "Kinetic and Mechanistic Studies of Signal Peptidase – A New Target for the Development of Antibacterial Agents", Temple University, School, of Medicine, Department of Biochemistry, May, 2000.

34. "Drug Discovery for Neurodegerative Diseases at Harvard Medical School", Meeting of the Parkinson Study Group, Santa Fe, NM, November, 2001.

35. "High-Throughput Screening and Drug Discovery for Neurodegenerative Diseases", Neurology Grand Rounds at Rhode Island Hospital, Brown University, School of Medicine, Department of Neurology, May, 2002.

36. "High-Throughput Screening and Drug Discovery for Neurodegenerative Diseases", Meeting of the Parkinson's Study Group, San Diego, CA, May, 2002.

37. "Ensemble of Transition States. Role of Conformational Mobility in Enzyme Catalysis", 29th Reaction Mechanisms Conference, Columbus, OH, June, 2002.

38. "New Models for Drug Discovery: Charting the Evolving Relationship between the Public and Private Sectors" Panel discussion at the IBC Drug Discovery Technology Conferency, Boston, MA, August, 2002.

39. "The Role of Mechanistic Studies in the Design of Enzyme Inhibitors," session organizer for national meeting of the American Chemical Society, Boston, MA, August, 2002.

40. "A New Model for Drug Discovery", Massachusetts Biotechnology Council's Conference on Biotechnology Investment, Boston, MA, October, 2002.

41. "A New Model for Drug Discovery", The Cure Parkinson's Project Conference – Accelerating the Cure, Chacago, IL, December, 2002.

42. "A New Model for Drug Discovery", Partners Program in Neurodegenerative Diseases 6th Annual Colluquim – Current Approaches to Understanding Neurodegenerative Diseases, Boston, MA, March 21, 2003.

43. "The Final Frontier – Therapies to Treat Neurological Disorders", session organizer, BIO 2003, Washington, DC, June 22-25, 2003.

44. "A New Model for Academic-Based Drug Discovery", BIO 2003, Washington, DC, June 22-25, 2003.

45. "Misguided Folding and Degradation of Protein – New Opportunities for Therapeutics in Neurodegenerative Diseases", BIO 2003, Washington, DC, June 22-25, 2003.

46. "A New Model for Academic-Based Drug Discovery", Drug Discovery Technology World Congress, Boston, MA, August 10-15, 2003.

47. "A New Model for Academic-Based Drug Discovery", Fourth Annual Institute for the Study of Agining Investiagotor's Meeting, Teaneck, NJ, October 1-3, 2003.

Part III: Bibliography

Original Articles

1. Matta MS, Green CM, Stein RL, Henderson P. Acylation of subtilisin carlsberg by phenyl esters. J. Biol. Chem. 1976;251:1006-08.

2. Vogler EA, Stein RL and Hayes JM. Mechanism of formation of grignard reagents. J. Amer. Chem. Soc. 1978;100:3161-66.

3. Stein RL, Romero R, Bull HG, Cordes EH. A kinetic α-Deuterium isotope effect for the binding of purine nucleosides to calf spleen purine nucleoside phosphorylase. Evidence for catalysis by distortion. J. Amer. Chem. Soc.. 1978;100:6249-51.

4. Romero R, Stein RL, Bull HG, Cordes EH. Secondary α-Deuterium isotope effects for acid-catalyzed hydrolysis of inosine and adenosine. J. Amer. Chem. Soc. 1978;100:7620-23.

5. Stein RL, O'Brien JK, Irwin C, Hunter FE. Extension of the biologic half-life of nitroglycerin through inhibition of organic nitrate ester reductase activity. Biochem. Pharmacol. 1980;29: 1807-13.

6. Stein RL, Cordes EH. Kinetic α-Deuterium isotope effects for *E. coli* purine nucleoside phosphorylase catalyzed phosphorolysis of adenosine and inosine." J. Biol. Chem. 1981;256:767-72.

7. Stein RL. Analysis of kinetic isotope effects on complex reactions utilizing the concept of the vitrual transition state. J. Org. Chem. 1981;46:3328-30.

8. Stein RL. Separation of choline and acetylcholine by cation-exchange chromatography. J. Chromatog. 1981;214: 148-51.

9. Stein RL, Elrod JF, Schowen RL. Correlative variations in enzyme-derived structures of catalytic transition states. Implications for the catalytic strategy of acyl-transfer enzymes. J. Amer. Chem. Soc. 1983;105:2446-52.

10. Stein RL, Viscarello BR, Kusner EJ, Krell RD. Purification of human leukocyte elastase and cathepsin G by chromatography on immobilized soluble elastin. Preparative Biochem. 1983;13:57-67.

11. Stein RL. Catalysis by human leukocyte elastase. Identification of rate-determining steps and operation of the charge-relay system. J. Amer. Chem. Soc. 1983;105:5111-16.

12. Stein RL, Viscarello BR, Wildonger RA. Catalysis by human leukocyte elastase. II. Rate-limiting deacylation for specific p-nitroanilides and amides. J. Amer. Chem. Soc. 1984;106:796-98.

13. Stein RL, Fujihara H, Quinn DM, Fisher G, Kullertz G, Barth A, Schowen RL. Transition-site structural features for anilide hydrolysis from α-Deuterium isotope effects. J. Amer. Chem. Soc. 1984;106:1457-61.

14. Ratcliffe SJ, Young GT, Stein RL. Amino acids and peptides. Part 49. 2-Amino-4-(3-pyridyl) butyric acid and related peptides. J. Chem. Soc. Perkin Trans. I, 1985;1767-71.

15. Stein RL, Williams JC. Proteolytic inactivation of human leukocyte elastase. Experientia 1985;41:638-40.

16. Stein RL. Catalysis by human leukocyte elastase. III. Steady-state kinetics for the hydrolysis of p-nitrophenyl esters. Archives Biochem. Biophys. 1985;236;677-80.

17. Stein RL. Catalysis by human leukocyte elastase. IV. Role of secondary-subsite interactions. J. Amer. Chem. Soc. 1985;107;5767-575.

18. Stein RL. Catalysis by human leukocyte elastase. V. Structural features of the virtual transition state for acylation. J. Amer. Chem. Soc. 1985;107;6039-42.

19. Stein RL. Transition state properties for the association of α-l-protease inhibitor with porcine pancreatic elastase. J. Amer. Chem. Soc. 1985;107:6039-42.

20. Aharony D, Stein RL. Kinetic mechanism of guinea-pig neutrophil 5-lipoxygenase. J. Biol. Chem. 1986;261:11512-19.

21. Stein RL, Trainor DA. On the mechanism of inhibition of serine proteases by chloromethyl ketones. Biochemistry 1986;25:5414-19.

22. Stein RL, Strimpler AM, Hisorhi H, Powers JC. Catalysis by human leukocyte elastase. VI. Mechanistic insights into the dependence of Pl-specificity on subsite interactions. Biochemistry 1987;26:1301-05.

23. Stein RL, Strimpler AM, Hisorhi H, Powers JC. Catalysis by human leukocyte elastase. VII. The proton inventory as a mechanistic probe of substrate specificity. Biochemistry 1987;26:1305-14.

24. Stein RL, Strimpler AM. Catalysis by human leukocyte elastase. VIII. Aminolysis of acyl-enzymes by amino acid amides and peptides. Biochemistry 1987;26:2238-42.

25. Stein RL, Strimpler AM. Chymostatin is a slow, tight-binding inhibitor of chymotrypsin and cathepsin G. Biochemistry 1987;26:2611-15.

26. Aharony D, Stein RL. Mechanism of inactivation of 5-lipoxygenase by 5-HPETE. Prostaglandins 1987;33:85-100.

27. Stein RL, Strimpler AM, Viscarello BR, Wildonger RA, Mauger RC Trainor DA. Mechanism for the slow-binding inhibition of human leukocyte elastase by valine-derived benzoxazinones. Biochemistry 1987;26:4126-30.

28. Stein RL, Strimpler AM. Primary specificity of serine proteases determines the operation of the catalytic triad during hydrolysis of tetrapeptide-derived acyl-enzymes. J. Amer. Chem. Soc. 1987;109:4387-90.

29. Stein RL, Strimpler AM, Edwards PD, Lewis JJ, Mauger RC, Schwartz JA, Stein MA, Trainor DA, Wildonger RA Zohola MA. Mechanism of slow-binding inhibition of human leukocyte elastase by trifluoromethyl ketones. Biochemistry 1987;26:2682-89.

30. Stein RL, Strimpler AM. Catalysis by human leukocyte elastase. IX. pH-dependent change in rate-limiting step. J. Amer. Chem. Soc. 1987;109:6538-40.

31. Stein RL. Transition state structural features for the thermolysin-catalyzed hydrolysis N-(3-[2-Furyl]acryloyl)-Gly-LeuNH$_2$. J. Amer. Chem. Soc. 1988;110:7907-8.

32. Harrison R, Teahan J, Stein R. A semi-continuous, HPLC-based assay for stromelysin. Anal. Biochem. 1989;80:110-13.

33. Teahan J, Harrison R, Izquierdo M, Stein RL. Substrate specificity of human fibroblast stromelysin. Biochemistry 1989;28:8497-8501.

34. Harrison RK, Stein RL. Mechanistic studies of peptidyl prolyl cis-trans isomerase: evidence for catalysis by distortion. Biochemistry 1990;29:1684-89.

35. Harrison RK, Stein RL. Substrate specificities of the peptidyl prolyl cis-trans isomerase activities of cyclophilin and FK-506 binding protein: evidence for the existence of a family of distinct enzymes. Biochemistry 1990;29:3813-16.

36. Izquierdo M, Stein RL. Mechanistic studies of thermolysin. J. Amer. Chem. Soc. 1990;112:605-62.

37. Harrison RK, Caldwell CG, Rosegay A, Melillo D Stein RL. Confirmation of the secondary deuterium isotope effect for the peptidyl prolyl cis-trans isomerase activity of cyclophilin by a competitive, double-label technique. J. Amer. Chem. Soc. 1990;112:7063-64.

38. Williams JC, Stein RL, Strimpler AM, Reaves B, Krell RD. Biochemical and pharmacological characterization of ICI 186,756: a novel, potent, and selective inhibitor of human neutrophil elastase. Exp. Lung Res. 1991;17:735-41.

39. Williams JC, Falcone RC, Knee C, Stein RL, Strimpler AM, Reaves B, Giles RE, Krell RD, Biologic characterization of ICI 200,880 and ICI 200,355, novel inhibitors of human neutrophil elastase. Am. Rev. Respir. Dis. 1991;144:875-83.

40. Williams JC, Stein RL, Giles E, Krell RD. Biochemistry and pharmacology of ICI 200,880, a synthetic inhibitor of human neutrophil elastase. Annals N.Y. Acad. Sci. 1991;624:230-43.

41. Izquierdo-Martin M, Stein RL. Mechanistic studies on the inhibition of thermolysin by a peptide hydroxamic acid. J. Amer. Chem. Soc. 1992;114:325-31.

42. Izquierdo-Martin M, Stein RL. Transition state structural features for the association of metalloproteinases with phosphorous containing inhibitors. J. Amer. Chem. Soc. 1992;114:1527-28.

43. Harrison RK, Stein RL. Enzymic and non-enzymic prolyl isomerization. J. Amer. Chem. Soc. 1992;114:3264-71.

44. Harrison R, Chang B, Niedzwiecki L, Stein RL. Mechanistic studies on the human metalloproteinase stromelysin. Biochemistry 1992;31:10757-62.

45. Niedzwiecki L, Harrison R, Teahan J, Stein RL. Substrate specificity studies for the human metalloproteinase stromelysin. Biochemistry 1992;31:12618-23.

46. Izquierdo-Martin M, Stein RL. Mechanistic studies on the inhibition of stromelysin by a peptide phosphonamidate. Bioorganic and Medicinal Chemistry 1993;1:19-26.

47. Chapman KT, Kopka IE, Dutette PL, Esser CK Lanza TJ, Izquierdo-Martin M, Niedzwiecki L, Chang B, Harrison RK, Kuo DW, Lin TY, Stein RL, Hagman WK. Inhibition of matrix metalloproteinases by N-carboxylalkyl peptides. J. Med. Chem. 1993;36:4293-4301.

48. Goulet JL, Kinnerary JF, Durette P, Hagmann WK, Stein RL, Harrison RK, Martin M, Kuo DW, Lin TY. Structure-activity relationships of phosphinic acid containing peptide inhibitors of stromelysin. Bioorg. Med. Chem. Lett. 1994;4:1221-24.

49. Izquierdo-Martin M, Chapman KT, Hagmann WK, Stein RL. Studies on the kinetic and chemical mechanism of inhibition of stromelysin by an N-(Carboxyalkyl) dipeptide. Biochemistry 1994;33:1356-65.

50. Stein RL, Izquierdo-Martin M. Thioester hydrolysis by matrix metalloproteinases. Archives of Biochemistry and Biophysics 1994;308:274-77.

51. Rock KL, Gramm C, Rothstein L, Clark K, Stein RL, Dick L, Hwang D, Goldberg AL. Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC-class I molecules. Cell 1994;78:761-71.

52. Esser CK, Kopka IE, Durette PL, Harrison RK, Niedzwiecki LM, Izquierdo-Martin M, Stein RL, Hagman WK. Inhibition of matrix metalloproteinases by N-carboxyalkyl peptides containing extended alkyl residues at P_1. Bioorg. & Med. Chem. Lett. 1995;5:539-42.

53. Stein RL, Chen Z, Melandri F. Kinetic studies of isopeptidase T: Modulation of peptidase activity by ubiquitin. Biochemistry 1995;34:12616-23.

54. Chapman T, Wales J, Sahoo SP, Niedzwiecki LM, Izquierdo-Martin M, Harrison RK, Stein RL, Hagmann WK. Inhibition of matrix metalloproteinases by P1 substitured N-carboxylalkyl dipeptides. Bioorg. Med. Chem. Lett. 1996;6:329-32.

55. Dick L, Cruikshank A, Grenier L, Melandri F, Nunes S, Stein RL. Mechanistic studies on the inhibition of the proteasome by lactacystin. A central role for *clasto*-Lactacystin ß-Lactone. J. Biol. Chem. 1996;271:7273-76.

56. Stein RL, Dick L, Melandri R. Kinetic characterization of the xhymotryptic ctivity of the 20S Proteasome. Biochemistry 1996;35:3899-908.

57. Melandri F, Grenier L, Plamondon L, Huskey P, Stein RL. Kinetic mechanism of the slow- and tight-binding inhibition of isopeptidase T by ubiquitin aldehyde. Biochemistry 1996;35:12893-900.

58. Dick L, Cruikshank A, Destree AT, Grenier L, McCormack TA, Melandri F, Nune, S, Palombella VJ, Parent LA, Plamondon L, Stein RL. Mechanistic studies on the inactivation of the proteasome by lactacystin in cultured cells. J. Biol. Chem. 1997;272:182-88.

59. McCormack T, Baumeister W, Grenier L, Moomaw C, Plamondon L, Pramanik B, Slaughter C, Souc, F, Stein RL, Zuhl F, Dick L. Active site directed inhibitors of *Rhodococcus* 20S proteasome: kinetics and mechanism. J. Biol. Chem. 1997;272:26102-109.

60. McCormack T, Dick L, Nunes S, Melandri F, Grenier L, Plamondon L, Stein RL. Proteasomes: activities and active sites. Protein Eng. 1997;10 Suppl 12.

61. Dang L, Melandri F, Stein RL. Kinetic and mechanistic studies on the hydrolysis of ubiquitin C-Terminal 7-Amido-4-Methylcoumarin by deubiquitinating enzymes. Biochemistry 1998;37:1868-79.

62. Palombella V, Conner E, Fuseler J, Destree A, Davis J, Laroux S, Wolf R, Huang J, Brand S, Elliott P, Lazarus D, McCormack T, Parent L, Stein RL, Adams J, Grisham M. Role of the proteasome and NF-κB in streptococcal cell wall-induced polyarthritis. Proc. Natl. Acad. Sci. U.S.A. 1998;95:15671-76.

63. McCormack T, Cruikshank A, Grenier L, Melandri F, Nunes S, Plamondon L, Stein RL, Dick L. Kinetic studies of the branched chain amino acid preferring peptidase activity of the 20S proteasome: development of a continuous assay and inhibition by tripeptide aldehydes and clasto-Lactacystin β-Lactone. Biochemistry 1998;37:7792-7800.

64. Adams J, Behnke M, Chen S, Cruickshank A, Dick A, Grenier L, Klunder J, Ma Y-T, Plamdon L, Stein RL. Potent and selective inhibitors of the proteasome: dipeptidyl boronic acids. Bioorg. Med. Chem. Lett. 1998;8:333-38.

65. Stein RL, Barbosa M, Bruckner R. Kinetic and mechanistic studies of signal peptidase I from *E. coli*. Biochemistry 2000;39:7973-83.

66. Stein RL, Nelson D, Thomas B. Slow-binding inhibition of γ-glutamyl transpeptidase by γ-boroGlu. Biochemistry 2001;40:5804-11.

67. Thomas B, Wang Y, Stein RL. Kinetic and mechanistic studies of penicillin binding protein 2x from *Streptococcus pneumoniae*. Biochemistry 2001;40:15811-23.

68. Stein RL. Enzymatic hydrolysis of *p*-nitroacetanilide. Biochemistry 2002;41:991-1000

69. Schwartz B, Markwalder JA, Seitz SP, Wang J, Stein RL. A kinetic characterization of the glysocyltransferase activity of *Eschericia coli* PBP 1b and development of a continuous assay. Biochemisty 2002;41:12552-12561.

70. Kosik KS, Ahn J, Stein RL., Yeh L-A. Discovery of compounds that will prevent tau pathology. J. Mol. Neurosci. 2003;19:261-266.

71. Case A, Stein RL. Mechanistic origins of the substrate selectivity of serine proteases. Biochemistry 2003;42:3335-3348.

72. Case A., Huskey W.P., Stein RL. Enzymatic reaction of silent substrates – kinetic theory and application to the serine protease chymotrypsin. Biochemistry 2003;42:4727-4732.

73. Case A, Stein RL. Kinetic analysis of the action of tissue transglutaminase on peptide and protein substrates. Biochemistry 2003;42:9466-9481.

74. Bruckner RC, Gunyuzlu PL, Stein RL. Coupled kinetics of ATP and peptide hydrolysis by E. coli FtsH protease. Biochemistry 2003;42:10843-10852.

75. Liu Y, Lashuel HA, Sungwoon C, Xing X, Case A, Ni J, Cuny GD, Stein RL, Lansbury PT Jr. Small-molecule inhibitors uncover antiproliferative UCH-L1 activity in one type of lung cancer. Chem. Biol. 2003;10:837-846.

76. Leissring MA, Lu A, Condron MM, Teplow DB, Stein RL, Farris W, Selkow DJ. Kinetics of amyloid β-protein degradation determined by novel fluorescence- and fluorescence polarization-based assays. J. Biol. Chem. 2003;278:37314-37320.

77. Wang Y, Bruckner R, Stein RL. Regulation of signal peptidase by phospholipids in membranes: characterization of phospholipid bilayr incorporated *Escherichia coli* signal peptidase. Biochemistry 2004;43:265-270.

78. Hengg AC, Stein RL. Role of conformational mobility in enzyme catalysis – Acylation of α-Chymotrypsin by specific peptide substrates. Biochemistry 2004;43:742-747.

79. Ahn JS, Musacchio A, Mapelli M, Ni J, Scinto L, Stein RL, Kosik K, Yeh L-A. Development of an assay to screen for inhibitors of tau phosphorylation by cdk5. J. Biomolecular Screening 2004;9:122-131.

Review Articles

1. Stein RL, Quinn DM, Schowen RL. Enzymatic strategies for the generation of catalytic power. In: Barth A, Haschen R, Laibach Possin H, Schowen RL, editors. Molecular and cellular regulation of enzyme activity; 1984. p. 1-25.

2. Stein RL, Trainor DA, Wildonger RA. Neutrophil elastase. Ann. Reports Med. Chem. 1985;20:237-46.

3. Stein RL. Exploring the catalytic activity of immunophilins. Current Biology 1991;1:234-236.

4. Stein RL. Mechanisms of enzymic and non-enzymic prolyl isomerization. Adv. Prot. Chem. 1993;44:1-24.

5. Goldberg A, Stein R, Adams J. New insights into proteasome function: from archaebacteria to drug development. Chemistry & Biology 1995;2:503-08.

6. Stein RL, Adams J. Novel inhibitors of the proteasome. Ann. Reports Med. Chem. 1996;31:279-88.

7. Stein, RL. A New Model for Drug Discovery. Drug Discovery Today 2003;8:245-248.

8. Stein, RL. High-Throughput Screening in Academia – The Harvard Experience. J. Biomol. Screen. 2003;8:615-619.

Thesis

The kinetic α-deuterium isotope effect as a probe of transition state structure and reaction mechanism in nucleoside hydrolysis [dissertation]. Indiana Univer.; 1978. Department of Chemistry, Laboratory of Professor Eugene H. Cordes.

Patents

1. Dutta SA, Stein RL, Trainor DA, Wildonger RA inventors; Proline derivatives as inhibitors of leukocyte elastase. European patent EP 124317 AC 1985.

2. Dutta SA, Stein RL, Trainor DA, Wildonger RA. Proline derivatives as inhibitors of leukocyte elastase. US patent 4,596,789. 1986 Jun 24.

3. Williams J, Glass M, Stein RL. New clinical use of the human neutrohil elastase inhibitor, ICI 220,880. US patent Application Filed 1990 May 24.

4. Stein RL, Ma Y-T, Brand S. Inhibitors of the 26S proteolytic complex and the 20S proteasome contained therein. International Patent Application, WO 95/24914, 1995 Mar 15.

5. Adams J, Ma Y-T, Stein RL, Baevsky M, Grenier L, Plamondon L. Preparation of peptidylboronic ester and acid compounds as proteasome inhibitors. US patent Application 95-549318. 1998.

6. Adams J, Ma Y-T, Stein RL, Baevsky M, Grenier L, Plamondon L. Boronic Esters and Acid Compositions. Synthesis and Uses. US Patent US 6,548,668 B2. 05/15/2003.

7. Adams J, Ma Y-T, Stein RL, Baevsky M, Grenier L, Plamondon L. Boronic Esters and Acid Compositions. US Patent US 6,617,317 B1. 09/09/2003.

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Atty. Docket No.	Serial Number	Description	Atty.	Fee
CLON-015	09/440,829	Transmittal, Fee Sheet *in duplicate,* IDS, SB08A, (5) Cited References	BEF	$180
CRMD-006	10/792,657	Transmittal, Fee Sheet *in duplicate,* Copy of Notice to File Missing Parts, Supplemental ADS, Executed Declaration, Formal Drawing Transmittal, (14) Sheets of Figures	CML	$603
UCAL-243	10/723,589	Transmittal, Fee Sheet *in duplicate,* Petition for a 3 Month Extension of Time, Supplemental ADS, Executed Declaration	KYB	$2,338
RIGL-005CON	10/057,467	Transmittal, Fee Sheet *in duplicate,* Amendment After Final Rejection, (2) Terminal Disclaimers, SB08A	JSK	$110
LBLB-002CIPCON2	10/155,918	Transmittal, Fee Sheet *in duplicate,* Preliminary Amendment	KYB	$180
CNVG-004US1DIV	09/721,158	PTOL-85B *in duplicate,* Formal Drawing Transmittal, (70) Sheets, Amendment 1.312	CML	$665
AGYT-022	10/795,148	Transmittal, Fee Sheet *in duplicate,* Supplemental ADS, Copy of Notice to File Missing Parts, Executed Declaration, Paper Copy of Seqlist, Seqlist Certification, (1) CD w/Seqlist in CRF	PJS	$65
CRMD-007	10/792,684	Transmittal, Fee Sheet *in duplicate,* Copy of Notice to File Missing, Parts, Supplemental ADS, Executed Declaration, Formal Drawing Transmittal, (13) Sheets of Figures	CML	$567
LIFE-018DIV	10/861,275	IDS, SB08A, Copies of SB08A from Parent, (5) cited References	CML	
UCAL-161DIV	09/922,483	Transmittal, Amendment After Final Rejection, Executed Declaration of R. Stein (Exhibits 1-2)	PAB	
LIFE-094	09/264,786	Transmittal, Fee Sheet *in duplicate,* PTOL-85B, Notification of Change of Entity Status, (7) Sheets of Figures, IDS, SB08A, (1) Cited Reference	CML	$1,510